The role of the qft-plus assay for eptb

Global new case notification of Extrapulmonary tuberculosis (EPTB) in 2005 was 0. 651 million which figured out at nearly one million at 20162 maintaining a steady increase within this era (2005-2016) and new case notification rate is considerably lower than the expected incidence rate according to World Health Organization (WHO) global tuberculosis report where misdiagnosed EPTB cases are remarkable factor. The Mycobacterium tuberculosis (Mtb) affects different parts of the body rather lungs in EPTB and most commonly involved the pleura, lymph nodes, abdomen, growing end of bones and joints, skin, genitourinary tract, meninges etc. with asymptomatic manifestation. EPTB is a burden with 15% cases among the 10. 4 million detected TB cases2 globally.

Diagnosis of EPTB remains challenging due to its paucibacillary nature, difficulties in obtaining proper specimens3 and similarities of its clinical presentation to other diseases4. Still, histopathology is on the frontline for EPTB diagnosis in Bangladesh. The histological similarity between cutaneous tuberculosis and other infectious granulomatous diseases, sarcoidosis, and granulomatous rosacea makes it awkward diagnosis solely from histopathology5. Culture is considered as the gold standard test for TB diagnosis and was found positive in 59% of reported EPTB cases as well as it is time-consuming and requires biosafety laboratory facilities 6, 7. GeneXpert MTB/RIF assay (Xpert) is recommended by WHO and this molecular test showed sensitivities of 100% on smear positive and 48% on smear negative EPTB cases8. However, above methods require an invasive procedure to collect the proper specimen and also the sensitivity varies with specimen type.

Quantiferon (QFT), an Interferon Gamma Release Assays (IGRAs) can make a remarkable footstep here because it is a test for cell-mediated immune (CMI) response to detect and quantify the interferon gamma (IFN-γ) which is released after Mtb infection 11. Quantiferon TB Gold Plus (QFT-Plus) assay, a new generation of QFT has been launched by QIAGEN®. The simulated peptide cocktail of ESAT-6 and CFP-10 stimulate cells in heparinized whole blood and interferon-γ (IFN-γ) associated with MTB infection is released after stimulation which is measured by enzyme-linked immunosorbent assay (ELISA) 12. This QFT-Plus assay is designed to draw CMI response from both CD4+ T-helper lymphocytes and CD8+ cytotoxic T lymphocytes; where ESAT-6 and CFP-10 specific CD8+ T lymphocytes are more frequently detected in subjects with active TB disease versus latent TB infection so it might be a useful marker for EPTB disease diagnosis and anti-TB treatment outcome monitoring. 13 Until this study has been conducted, it was anonymous that the performance of the QFT-Plus as a tool to monitor anti-TB treatment. The previous generations of QFT had been discouraged for diagnosis of TB in low and middle income country, 15 nevertheless with the added advantage of CD8+ the new generation QFT-Plus should be studied in this region for the faster and easy diagnosis of TB, particularly for the diagnosis complications of EPTB.

In this study, we aimed to investigate the role of the QFT-Plus assay for EPTB case identification and treatment outcome monitoring of EPTB patients because control and prevention of tuberculosis depend on successful completion of treatment16. The study was funded by Sida (The Swedish International Development Cooperation Agency) and the protocol was

reviewed and approved by the Research Review Committee (RRC) and the Ethical Review Committee (ERC) of icddr, b. The study participants were enrolled from Dhaka Medical College and Hospital which is a large tertiary public health care facility in Bangladesh where patients from different areas of Bangladesh are referred here for better management. An RRC and ERC approved written informed consent was also obtained from eligible interviewed participants. Patient with presumptive extrapulmonary whose specimens was available for bacteriological investigations was the inclusion criteria and patient with pulmonary TB or under anti-TB therapy was excluded from the study. The extrapulmonary specimens were transported in cool box and blood specimens were transported in ambient (22+/- 5 °C) temperature to Mycobacteriology Laboratory, icddr, b after collecting from enrolled participant.

The following extrapulmonary specimens were collected from presumptive EPTB cases: Ascitic fluid (AF), pleural fluid (PF), cerebrospinal fluid (CSF), pus, synovial fluid, urine, tissue, lymph node aspirate, Fine-needle aspiration biopsy (FNAB) specimen etc. Laboratory tests for extrapulmonary specimens:

The extrapulmonary specimens were directly subjected for Xpert (Xpert MTB/RIF assay) test for molecular detection and later at the same day processed through the N-acetyl-L-Cystein (NALC) and Sodium hydroxide (NaOH) method (NALC-NaOH method) as described previously17 and inoculated on both LJ media and BACTEC MGIT 960 (Becton Dickinson, Sparks, MD) according to the manufacturer’s instructions18 for phenotypic detection. Only CSF specimens were directly inoculated on LJ media and

BACTEC MGIT 960. Quantiferon-TB Gold Plus assay (QFT-Plus) test for Blood specimen:

The first blood specimen was collected for each participant during enrollment and the second blood was collected from those participants who continued for anti-TB treatment, after six months of the starting date of anti-TB treatment. Blood Specimens were incubated at 37 +/- 1 °C for 16–24 h within 16 h after collection and after incubation the tubes were centrifuged at 3000 × g for 15 min. The test was done within one week of collection and left over plasma (250 µL) was stored at − 80 °C after harvesting on microcentrifuge tube. The standard QFT-Plus ELISA kit was used for ELISA, the optical density of each well will be measured on a plate reader (MRXe Dynex, Magellan Biosciences) using the QFT-Plus analysis software (Software Version 2. 71, Qiagen). The concentration of released IFN-γ in each tube was calculated by subtracting the value of the nil (negative control) tube. If the coefficient of variation for the result was less than 15% and the Correlation coefficient for the standard curve was greater than 0. 98, the assay was considered to be technically valid. All of the results were interpreted by referring to a 4-point standard curve.

The ELISA results of the QFT-Plus test was interpreted as follows. A positive test was defined as antigen − nil ≥ 0. 35 IU/mL and ≥ 25% of the nil sample, where as a negative test was defined as antigen–nil < 0. 35 IU/mL or < 25% of nil, when mitogen ≥ 0. 5 IU/mL. The results was considered indeterminate if 1) nil > 8 IU/mL or 2) antigen–nil ≥ 0. 35 IU/mL and < 25% of nil when the nil was ≤ 8. 0 IU/mL and the mitogen response was < 0. 5 IU/mL.

Result:

A total of 203 participants were enrolled in this study as presumptive EPTB patient and two of them were excluded after pulmonary TB (PTB) coinfection confirmation. Among the 201 participants, 66 (32. 83%) started anti-TB therapy as EPTB patient and 50 patients were available for follow up after six months. The table 1 depicts the demographic data, specimen type and detected EPTB case from the enrolled study participants. The participants were assembled in three age groups, nineteen participants were from 0-17 years aged group (mean age 14. 26 years, SD ±1. 4), one hundred and forty seven participants were from > 17-64 years aged group (mean age 38. 46 years, SD ±13. 9) and 35 participants were from > 64 years aged group (mean age 71. 42 years, SD ±6. 2). Sensitivity and specificity of Culture: The culture sensitivity and specificity was 25. 75% and 97. 32% considering clinical diagnosis as reference standard.

Sensitivity and specificity of Xpert:

Considering culture as reference standard for bacteriological identification, the sensitivity and specificity of Xpert was 64% and 98. 86%. Furthermore, the sensitivity and specificity was 23% and 99. 21%, respectively when the composite standard (Clinical identification + Culture confirmed) was considered as reference standard.

Sensitivity and specificity of QFT-Plus:

QFT-Plus cannot differentiate between active TB disease and latent infection but it can confirm Mtb infection. In this respect, we calculated the sensitivity and specificity of QFT-Plus for EPTB diagnosis compared to culture and true disease (patient who completed anti-TB treatment and diagnosed by

bacteriologically and/or clinically). The sensitivity and specificity of QFT-Plus compared to culture is 54. 54% and 74. 23%. In this respect, the composite of bacteriological positive and clinically diagnose positive was considered as reference standard for QFT-Plus test accuracy determination and the indeterminate results were excluded from accuracy analysis. 19 The calculated EPTB disease prevalence was 36. 61% in this study setting. The sensitivity, specificity, positive predictive value, negative predictive value, positive likelihood ratio, and negative likelihood ratio was 52. 24% (95% Confidence interval, CI, 40. 28%- 72. 2%), 84. 5% (95% CI, 78. 07%-90. 9%), 66% (95% CI, 55%-76%), 75. 38% (95% CI, 65. 06%-85. 69%), 3. 37 (95% CI, 2. 08-5. 45), and 0. 57 (95% CI, 0. 43-0. 73), respectively. The calculated accuracy for all EP specimen and for specific EP site for example CSF, AF, PF is depicted in table 2. The specificity for tuberculous meningitis (CSF specimen) was 76. 92% and it was 100% for tuberculous pleurisy (Pleural fluid). The sensitivity was also much lower for tuberculous meningitis (38. 10%) patient than the tuberculous pleurisy (58. 62%) or tuberculous peritonitis (53. 85%). Diabetic patient and QFT-Plus result: Among the 73 composite positive patient, twelve patients were diabetic according to patients self-report; where 7 of them were positive, four negative and one indeterminate by QFT-Plus assay. In our setting diabetes was not responsible for poor performance of the QFT-Plus among the EPTB cases ($p > 0. 05$).

Effect of previous active TB disease:

Among the 201 enrolled participant 16 had previous TB where 6 of them had PTB, 10 had EPTB. Fourteen patients were cured after completion of their treatment and two patient did not complete their treatment; these two

patients finally did not diagnosed as EPTB case in this study. Two of previously diagnosed PTB became EPTB case as one TM and one TPE; one had TP who could not define his previous EPTB type. The previous TB history did not have any association for development of EPTB disease (Odds ratio, OR 0. 379, 95% CI, 0. 104- 1. 377; p > 0. 05). 56. 2% of previously diagnosed patient had negative QFT-Plus result during the time of enrolment in this study. Other disease diagnosis among the presumptive EPTB patient:

As mentioned above, among the 203 participants, 2 participants were excluded since they were pulmonary TB and extrapulmonary TB co-infected. Sixty-six patient started anti-TB treatment within 14 (0-14) days of presumption, based on either clinical, biochemical (Adenosine deaminase) or bacteriological confirmation. The final diagnosis among the presumptive EPTB patient after excluding EPTB suspicion were cancer, liver cirrhosis, inflammation, gastric ulcer, Hippocampal sclerosis, Wilson disease, Hepatitis B, Lymphadenitis etc. Among the confirmed non-tuberculosis diagnosed patients (n= 111), fourteen (12. 6%) patients were positive and eleven (9. 9%) were indeterminate on QFT-Plus test; the vast majority (77. 5%) were negative on QFT-Plus test from this group.

Discussion:

Some diseases management depends on diagnostic strategy rather single test where EPTB can be a paramount example. 20 This prospective study was done to understand the kinetic of IFN-Gama responses for EPTB cases and to determine the usefulness of IGRA test as diagnostic tool during EPTB anti-TB therapy. This prospective study was done to understand the usefulness of updated version of Quantiferon- the QFT-Plus assay as

diagnostic tool for EPTB diagnosis. Fairly focusing on a particular populations, we investigated the study in various risk populations referred according to present national guidelines21 and thus reflecting the clinical situation with the socio-demographic data at the large Bangladeshi tertiary public hospital. This is the best of our knowledge that this study is the first to associate the practicality of QFT-Plus and other methods for diagnosing EPTB in Bangladesh. The accuracy of the QFT-Plus test vary for site of tuberculosis infection and it is lower for Tuberculosis meningitis (59. 57%, 95% CI, 44. 27% – 73. 63%), it may be due to the poor immune condition of the tuberculous meningitis patient and therefore for the tuberculous meningitis diagnosis from blood QFT-plus might not be advantageous. However, over all accuracy of QFT-Plus from all type of EPTB patient is 72. 68% (95% CI, 65. 61% – 78. 99%). One drawback of QFT-Plus is its test result may vary depending on patient response to TB antigen; indeterminate result for example adds no significance for TB patient management and false negative result leads to delayed or erroneous diagnosis.

There are contradictory data on QFT performance among the tuberculosis patient with diabetes. Walsh et al. reported IGRAs is not compromised in tuberculosis patient with diabetes, on the other hand Faurholt-Jespen, D. et al. reported lower antigen response in tuberculosis and non-tuberculosis control; 24, 25 however in this study setting QFT-Plus performance was not hampered by the diabetic condition among the EPTB patient ($p > 0. 05$). Previously diagnosed TB, those who were cured after treatment at least one year before, previous TB history did not play as factor for their recent TB disease (OR 0. 379, $p> 0. 05$), though we had only new 3 EPTB cases from

the 16 previously diagnosed TB out of 201 participants, may be a large sample size of this type may better correlate the previous TB history and the EPTB disease. Moreover, only 5 patient had positive QFT-Plus result, 2 had indeterminate QFT-Plus result and 9 had negative QFT-Plus result during the time of enrollment period (table 2). Both the indeterminate resulted patient had chronic kidney disease which is a factor for poor performance of IGRA test; 26, 27 among the negative resulted 9 patients one had recent TM diagnosis and other 8 had cancer or non-TB diagnosis; these patient might had erase of TB related IFN-G signature due to cured after treatment or false negative result for their immunosuppressive condition.

Study from developed country like the United Kingdom of Denmark suggested QFT can greatly improve the diagnosis of EPTB but it is well established that QFT accuracy depends on geographical region. 30, 31 Many biological factor or intrinsic design of the assay might have impact on the sensitivity of QFT-Plus. 32 The sensitivity (52. 5%) and specificity (84. 48%) data from this study depicts that in high TB burden region like Bangladesh, QFT-plus may not aid better service for inclusion or exclusion from EPTB presumption. Again it should be considered that there is possibility of false negative result for poor immune status of the individual as EPTB patients are immunologically feeble in general. There are some limitations of this study. First we did not account the Tuberculin Skin-test (TST) to compare with QFT-Plus assay but it is worth noticing that QFT offers an improvement over TST by single visit with superior performance and to reduce patient knuckle for second visit, TST was not considered in this study.

To achieve the ‘ End TB Strategy’ as well as to reduce the morbidity of EPTB patient and EPTB mirroring diseased patient, improved technique with new biomarker can be investigated for better performance of QFT-Plus for the endemic region like Bangladesh.